**Effect of Lethal and Sub-lethal Concentrations of Heavy Metal (Zinc) on Opercular Beat Frequency (OBF) in *Cyprinus carpio* var. *communis***

**Abstract**

In the present investigation,the acute and sublethal toxicity of a grey listheavy metal (Zinc) on the juvenile of Common carp (*Cyprinus carpio*)was evaluated to determine its effect on the Opercular beat frequency (OBF) under laboratory conditions. The fish was exposed to varying levels of the toxicant (2.12mg/L; 4.25mg/L and 8.50 mg/L) for 96 hrs using static bioassay. Opercular beat frequency (OBF) in exposed fishes initially increased at 6 hr and 12 hr time interval compared to the control followed by a gradual decrease at later time points (48, 72 and 96 hour time intervals) that reflected a mild inhibitory effect. The operculum beat frequency was significantly (P< 0.05) affected by the exposure and concentration indicatingan impaired respiratory function. A decline or alteration in OBF suggests respiratory stress, which is a direct marker of toxic effects. This study gives clear insight into how zinc toxicity impair basic life-sustaining functions in fishes. These findings highlight the potential of opercular beat frequency as a sensitive bioindicator for assessing sublethal respiratory stress in fish exposed to heavy metal contaminants like zinc.

**Key words**

Zinc, Heavy metal, Opercular Beat Frequency, Carp, Toxicity, Lethal exposure

**Introduction**

Zinc is presumably an essential structural, catalytic and regulatory micronutrient for many enzymes and critical for protein synthesis, cell proliferation, growth, development and reproduction (Vallee and Falchuk, 1993; Hogstrand and Wood, 1996).However,on the other hand, it is toxic at elevated concentrations. Zinc may be transported to the aquatic ecosystems as a result of both natural and anthropogenic sources (Shu´illeabha´in *et al*., 2004). Fish takes in zinc through the gastrointestinal tract and the gills. Zinc can accumulate in tissues and organs of fishes living in zinc contaminated water resources. Behaviour is a sensitive measure of an organism’s response to stress including environmental contaminants. Change in behaviour can even be noticed when the fish is exposed to a chemical lower than those that cause mortality (Little and Finger, 1990; Scott and Sloman, 2004). Any change in fish behaviour provide valuable information and knowledge regarding behavioural alterations which can be related to physiological biomarkers in aquatic species (Hellou, 2011). Use of behavioural alterations in organisms in response to exposure to varying levels of pollutant is increasingly studied in ecotoxicology for improving the determination of ecologically relevant risk end point. It serves as biological indicator which provides a unique perspective linking the physiology and ecology of an organism and its environment. Therefore, in the present investigation, changes in opercular beat frequency of *Cyprinus carpio* var. *communis* exposed to lethal and sub-lethal concentrations of zinc were studied.

**Material and Method**

Healthy fingerlings of *Cyprinus carpio* var*. communis* weighing approximately 10 ± 2 gms, were procured from Nursery ponds at Faculty of Fisheries, Rangil, Ganderbal. Heavy metal, Zinc in the form of Zinc sulphate, was purchased from a local laboratory chemical dealer in Srinagar, Jammu and Kashmir. They were brought to laboratory in plastic bags with adequate water to avoid any physical injury. The fishes were disinfected by giving them a bath for two minutes in 0.05% KMnO4 solution. Thereafter, they were transferred to glass aquaria measuring 60×30×40 cm. Prior to the introduction of fishes, aquaria were also washed with KMnO4 solution to avoid any infection. Fishes were acclimatized for two weeks and fed with artificial diet during the period. Leftover food in the tank was removed daily and water was changed. Standard dilution water containing zinc sulphate as the toxicant was prepared according to OECD standards ([2019](https://link.springer.com/article/10.1007/s00128-018-2441-2#ref-CR24)) for experimentation.

Ventilatory activity in terms of opercular movement was measured manually by counting the number of times the operculum opened and closed per unit time with the help of a stopwatch and represented as opercular movements per minute. Opercular beat frequency in fishes subjected to different toxicant concentrations (2.12, 4.25 and 8.50 mg/L) were recorded after 6 hr, 12 hr, 24 hr, 48 hr, 72 hr and 96 hr. Fish used as control was monitored along with the toxicant concentrations to provide a reference for assessing any changes. Fishes were treated dead, if there was no opercular movement, immobilization, loss of equilibrium or morbidity as all these parameters indicated a pending death.

Statistical Analysis

Statistical analysis was performed in SPSS (20.0 version) using ANOVA (Analysis of Variance).

**Result and Discussion:**

The tested fishes were exposed 2.12 mg/l, 4.25 mg/l and 8.50 mg/l in order to assess Opercular beat frequency responses in juvenile *C. communis*. Each experimental parameter was tested in triplicate (R1, R2, and R3) to ensure accuracy and reliability. The results of the study are described.

The opercular beat frequency was measured across six different time intervals (6 hr, 12 hr, 24 hr, 48 hr, 72 hr, and 96 hr) for a control group and three treatment groups (T1, T2, T3). The opercular beat frequency varied across both time intervals and treatment groups. Opercular beat frequency in the Control group was relatively consistent over time, staying higher than the exposed groups at most time points which indicated a stable respiratory activity unaffected by external stressors. In group T1, opercular beat frequency was initially increased at 6 hr and 12 hr time interval compared to the control followed by a gradual decrease at later time points (48, 72 and 96 hour time intervals) that reflected a mild inhibitory effect. Group T2, showed a moderate increase in opercular beat frequency compared to the control at 6 hr, and then decrease especially noticeable from 24 hours onwards which indicated a stronger effect of this exposure level on respiratory activity. In group T3, opercular beat frequency started slightly lower or comparable to other groups at 6 hour time interval but declined significantly over time, particularly at 48, 72 and 96 hours and reflected a severe inhibitory effect of high exposure, likely indicating respiratory distress. Also, higher concentrations (4.25 mg/l and 8.5 mg/L) led to greater reductions in opercular beat frequency, which clearly showed a dose-dependent inhibitory effect. At 6 hour time interval, highest opercular beat frequency with an average of 81 movement/ minute was observed in group T1. Statistically significant differences in opercular beat frequency were found among the treatment groups and with control group at various time intervals (p< 0.05). These findings suggest that prolonged exposure leads to a dose-dependent decline of opercular movements, with all exposure groups showing significantly reduced opercular beat frequency (8.5 mg/l > 4.25 mg/l > 2.12 mg/l) compared to the control group, with variations observed over time and among treatment groups as shown in Table 1 and Figure 1.

**Table 1: Opercular beat frequency exhibited by the fish exposed to Zinc bioassays.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time** | **Control** | **T1**  | **T2**  | **T3**  | **Mean for time± S.E** | **P-value** |
| **6 hr** | 72.17±0.90 | 81.20±2.52 | 75.43±2.39 | 70.47±2.61 | 74.82±2.36 | 0.085 |
| **12 hr** | 66.13±1.02 | 70.73±0.44 | 65.50±2.38 | 54.47±1.82 | 64.21±3.45 | 0.079 |
| **24 hr** | 64.50±0.78 | 63.93±0.80 | 59.17±0.90 | 52.43±1.08 | 60.01±2.79 | 0.045 |
| **48 hr** | 73.50±3.08 | 56.67±2.28 | 50.77±1.45 | 44.30±1.54 | 56.31±6.26 | 0.031 |
| **72 hr** | 73.43±2.28 | 47.53±2.19 | 42.67±2.61 | 37.87±2.83 | 50.38±7.94 | 0.023 |
| **96 hr** | 76.90±4.05 | 40.73±3.58 | 35.33±3.68 | 31.87±3.76 | 46.21±10.39 | 0.041 |
| **Mean for concentration** | 71.11±1.95 | 60.13±6.10 | 54.81±6.06 | 48.57±5.60 |   |
| **P -value** | 0.66 | 0.011 | 0.062 | 0.034 |   |

**Figure 1: Opercular beat frequency in the control fishes and Zinc treated fishes exposed to 2.12 ppm, 4.25 ppm and 8.5 ppm concentrations at 6 hrs, 12 hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs. Data represent mean ±SEM, \*p<0.05, compared to control.**

**Discussion**

Opercular beat frequency (OBF) is a measure of how often a fish's operculum, or gill cover, moves.  In the present study an increase in OBF with respect to time and concentration of Zinc sulphate was noticed in *Cyprinus carpio* var. *communis.*  The increase in the opercular beats as a primary response to sudden stress was also reported by Rajasekaran *et al*. (2009). The increased opercular activity may be due to shock received by the fish in new toxic environment along with sensory stimulus to increase the opercular movement for proper ventilation of the gills to cope with hypoxia (Joseph *et al.* 1987; Lata *et al.* 2001). *Catla catla* on introduction to lethal concentration of copper showed abnormal behaviours such as rapid opercular movement such as behavioural changes could be due to osmatic imbalance which affects nervous system. The above symptoms of poisoning have also been investigated in *Clarias batrachus* when treated with malathion in *B. stigma* treated with carbaryl (Sharma and Aggarwal, 1996). In a study made by Sinha (2019), on opercular activity (number of beat / min) of zinc exposed *Catla catla*, OBF increased with increase in the concentrations of copper and duration of exposure. Joshi (2011) reported that *Clarias batrachus* in toxic media exhibited hypo and hyper opercular activity. Although the mode of function of these toxicants is markedly different than zinc sulphate but behavioural changes observed are similar to our study. The increased ventilation rate by rapid, repeated opening and closing of mouth and opercula coverings accompanied by partially extended fins (coughing) was observed in the present study. This could be due to clearance of the deposited mucus debris in the gill region for proper breathing (Prashanth *et al.,* 2005).

**Conclusion**

 In conclusion any change in Opercular Beat Frequency can be used as primary indicator of fish to stress and any kind of toxin exposure. OBF initially increased as the toxin was introduced in the media. However with longer duration of exposure, it gradually started to decrease. It also showed a declining trend with increase in concentration of Zinc. These studies are critical in understanding the fast impacts of heavy metal pollutants on fish populations, which serve as crucial indicators of water quality and ecosystem health. The results of the present study emphasize the need for careful heavy metal management to protect the freshwater biodiversity.

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